

10/031496

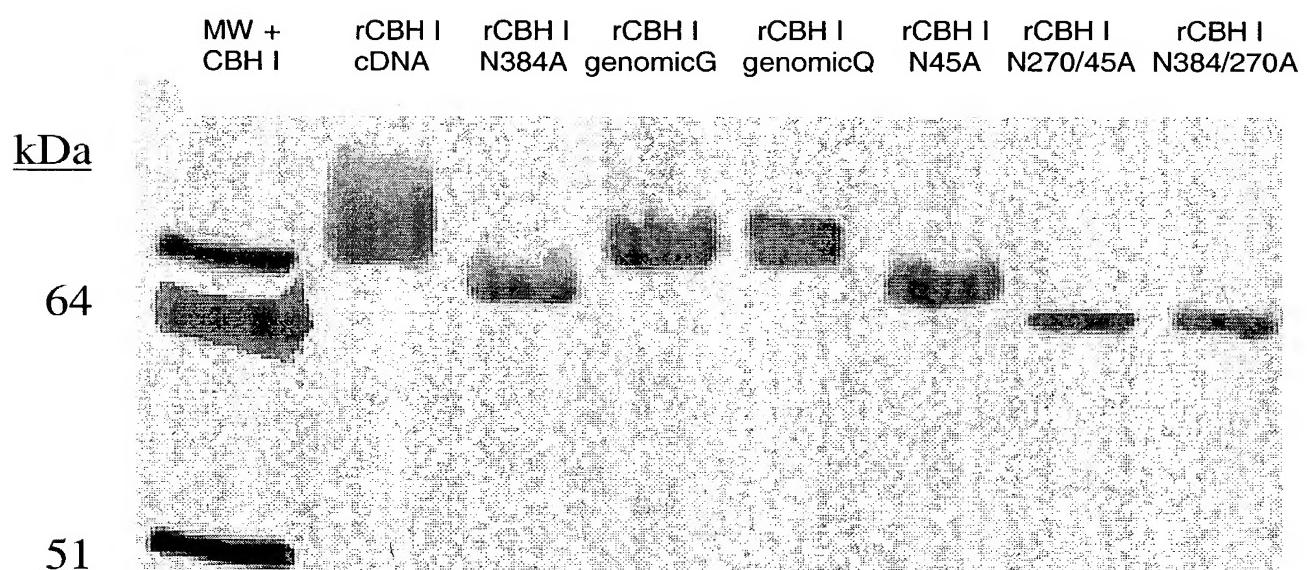
Figure 1. Coding sequence for the *cbh1* gene. Small case letters represent the signal sequence, large case letters the catalytic domain, bolded italics the linker region, and large case underlined the cellulose-binding domain.

5

atgtatcggaaagtggccgtcatctggcccttgcacagctgtgt**CAGTCGGCCTGCAC**TCTCCAATCGGA
GA**CTCACCCGCC**TCTGACATGGCAGAAATGCTCGTCTGGTGGCACGTGCAC**TAACA**CA
GACAGGCTCCGTGGT**CATCGACGCCA**ACTGGCGCTGGACTCACGTACGAACAGCAG
CACGA**ACTGCTACGATGGCAAC**ACTTGGAGCTCGACC**CTATGTC**CTGACAACGAGAC
CTGCG**GAAGAA**ACTGCTGTCTGGACGGT**GCC**CTACGCGT**CCACGTACGGAGTT**TAC
CACGAGCG**GTAA**CAGCCT**CTCCATTGGCTTGT**ACCCAGT**CTGCGCAGAA**AGAACGT
TGGCG**CTCGC**TTTAC**CTTATGGCGAGCGACACGAC**CT**ACCAGGA**ATT**CACCC**TG**GCTT**
GGCAAC**AGAGTTCTCTTCGATGTTGATGTT**CGAGCT**GCC**GT**CGGGCTT**GAACGG**A**
GCT**CTCTACTTCG**TGTCCATGGAC**CGGG**ATGGTGG**CGTGAGCAAGTATCCCACCAAC**
ACCG**GCTGGCGCC**AAGT**ACGGCACGGGG**ACT**GTGACAGCC**AGT**GTC**CCCC**CGC**GAT**CTG**
AAG**TTCATCA**ATGGCCAGGCCA**ACGTTGAGGG**CTGGAG**CCGT**CAT**CCAACAA**AC**GC**
GAAC**ACGGG**CATTGGAG**GAACCGGAAG**CTG**CTGCTGAGATGG**AT**ATCTGGGAGG**
CCA**ACTCC**AT**CTCCGAGG**CT**TTACCCCCCACC**CT**TG**CACG**ACTG**CG**GGCCAGG**GAGA
TCT**CGAGGG**TGAT**GGGTGCGG**GA**ACTTACTCC**GATA**ACAGA**T**ATGGCGG**ACTT
GCG**ATCCC**GAT**GGCTGCG**ACT**GGAACCC**AT**ACCGCTGG**CA**ACACCCAG**CTT**CTACG**
GCC**CTGG**CT**CAAG**CTT**ACCC**CT**CGATACCACCAAGAA**AT**GACCGT**GT**CACCC**AGT**TTCCAGCA**
CGAGAC**GT**CGGG**GTGCC**AT**CAACCG**AT**ACTATG**TC**CAGA**AT**GGCG**TC**ACTTCC**AG**CA**
GCC**CAACG**CC**GAG**CT**GGTAGTT**ACT**CTGG**CA**ACGAG**CT**CAACG**AT**GATT**ACT**GCAC**
AG**CTGAGGAGG**CAGA**ATT**CG**GGG**AT**CC**CT**CTT**CT**CAGACA**AGGG**CGGC**CT**GACT**CA
GTT**CAAGAAGG**CT**ACCT**CT**GGCGG**AT**GGT**CT**GGT**CAT**GAGT**CT**GTGGG**AT**GATTA**
CTACGCC**AA**AT**GCTG**GG**CTGG**ACT**CCAC**CT**ACCC**GAC**AAACG**AG**AC**CT**CC**CC**AC**
AC**CCGGT**GC**CGT**GC**CGG**A**AGCT**GT**CCACC**AG**CT**CC**GGT**GT**CCCT**G**CTCAGG**TC**GA**
AT**CTCAGT**CT**CCCAAC**CC**GAAG**GT**CAC**CT**CC**AA**ACAT**CA**AGTT**CG**GA**CC**CA**TT**GGC**
AG**CACCGG**CA**ACC**CT**AGCGG**CG**CAAC**CT**CC**CC**GGG**AA**ACCC**CC**CTGG**CA**CC**
AC**CCACCC**CC**GGCC****AGCC****ACTACC****ACTG****GAAG****CT****CTCC****GGAC****CT****ACCC****AGT****CTCA**
ACGGCC**AGT****GC****GGCG****GT****ATTGG****CTACAGCGG****CCCCACGG****CTGC****GCCAGCGG****CACA**
ACTT**GCCAGG****T****CTGA****ACCC****TTACT****ACTCTCAGT****GCCTGT****AAAGCT****CC**

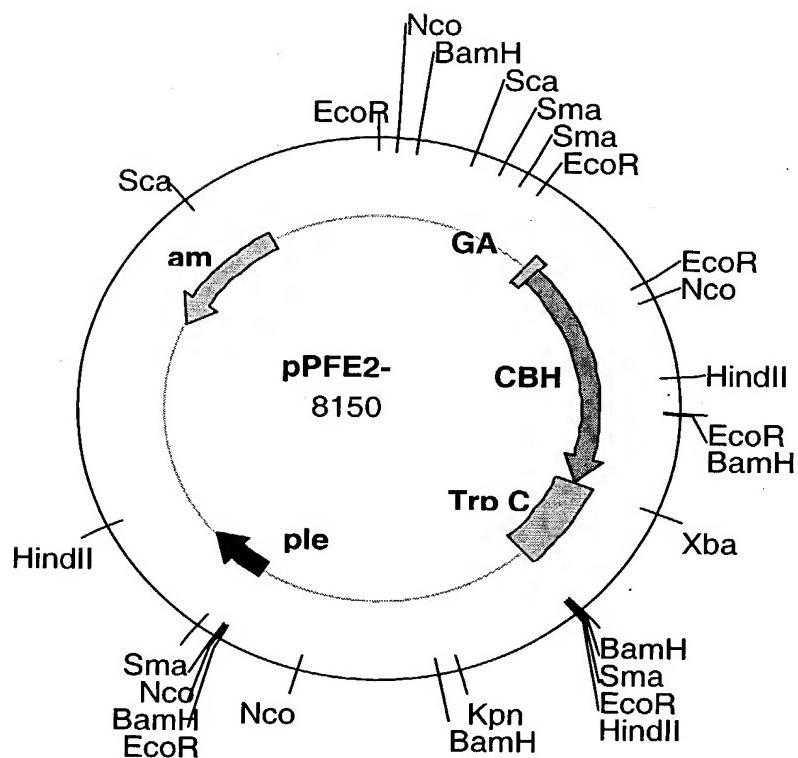
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Figure 2. SDS-PAGE western blot using anti-CBH I showing the reduction in molecular weight of rCBH I expression clones as a function of the introduction of N>A modifications.



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Figure 3. Plasmid map of fungal expression vector pPFE2/CBHI



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Figure 4. Coding sequence for the linker region showing additional proline that effect conformation of linker region in the protein structure.

P P G G N P P G T T T T R R P
CCTCCCGGCGGAAACCCGCCTGGCACCAACCACCCGCCGCCA
GGAGGGCCGCCTTGGCGGGACCGTGGTGGTGGCGGGCGGGT
0 20 40